

Application Serial No. 09/980,585
Amendment Dated 20 September 2004
Reply to Office Action of 4 May 2004

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims

1 (Currently amended): A method to enable the assessment of the growth rate and death rate of a micro-organism

within a chosen time period in an environment of interest by comprising introducing into said micro-organism at least two reporter genes, which method is characterised in that wherein
a) said reporter genes code for luminescent and/or fluorescent products and
within said time period and environment producing at least two said products of the following are produced:

i) a stable product produced in step (a), within the environment of interest, essentially known proportion to the total amount of cells of said microorganism that are or have been alive within said chosen time period,

ii) a product present in said environment of interest in an essentially known proportion to the amount of cells alive at any moment within said chosen time period, and

iii) a stable product produced in step (a), within the environment of interest, essentially known proportion to the total amount of cells of said micro organism that have died within said chosen time period, and said products can be measured through their luminescence and/or fluorescence;

b) incubating said micro-organism is incubated within the environment of interest and detecting said luminescence and/or fluorescence is detected after said chosen time period, and

c) assessing the growth and death rate of the said micro-organism is assessed based on measuring at least two of the following:

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- i) the known proportion of luminescence or fluorescence to the amount of cells alive after any said chosen time period,
- ii) the known proportion of luminescence or fluorescence to the total amount of cells that are or have been alive within any said chosen time period, and
- iii) the known proportion of luminescence or fluorescence to the total amount of cells that have died within any said chosen time period.

2 (Currently amended): The method according to claim 1 characterised in that wherein said micro-organism is a gramnegative bacteria, e.g. *Escherichia coli*.

- 3 (Currently amended): The method according to claim 1 characterised in that wherein
- a) one reporter gene coding for a luminescent product is luciferase, which is used for the determination of amount of cells alive at any moment within said chosen time period, and
 - b) another reporter gene coding for a fluorescent product is green fluorescent protein (GFP), which is used for the determination of total amount of cells of said micro organism that are or have been alive within said chosen time period.

4 (Currently amended): The method according to claim 1 characterised in that wherein said reporter genes are introduced into said micro-organism in a plasmid.

5 (Currently amended): The method according to claim 3 characterised in that wherein said plasmid is pGFP+luc* (SEQ ID NO: 1).

- 6 (Currently amended): The method according to claim 2 characterised in that wherein
- a) one reporter gene coding for a luminescent product is luciferase, which is

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used for the determination of amount of cells alive at any moment within said chosen time period, and

b) another reporter gene coding for a fluorescent product is green fluorescent protein (GFP), which is used for the determination of total amount of cells of said micro organism that are or have been alive within said chosen time period.

7 (Currently amended): The method according to claim 2 characterised in that wherein said reporter genes are introduced into said micro-organism in a plasmid.

8 (Currently amended): The method according to claim 4 characterised in that wherein said plasmid is pGFP+luc* (SEQ ID NO: 1).

9 (Currently amended): The method according to claim 6 characterised in that wherein said plasmid is pGFP+luc* (SEQ ID NO: 1).

10 (Currently amended): The method according to claim 7 characterised in that wherein said plasmid is pGFP+luc* (SEQ ID NO: 1).